## MICROSCOPY.[1]

**Notes on Gold Impregnation Technique.**—The following method of using formic acid and gold chloride is a modification, or adaptation of a method used by Miss Julia B. Platt and kindly suggested by her to me. She refers it to Professor Mark of Harvard University. I have used it in tracing the nervous system of *Nephelis lateralis* and have found it reliable. In leech tissues, it differentiates all nerve tissue, though the histology of other tissues is poor. After more than a year's use of this method without a complete failure among my preparations, I feel that Lee's characterization of the other methods of gold staining does not apply to this method.

It has been used successfully on larval vertebrate material as well as on leech tissue, by varying the strength of the formic acid, or the time of its application. The other factors are to a great extent indifferent as to strength used or time employed. If maceration occurs, lessen the action of the formic by weakening or by shortening the time. If the impregnation is slight, increase the action. The thickness of the piece stained should not exceed 5 mm., and the tissue must be living.

The following is the process employed with *Nephelis:*

The leech is put into twenty or thirty times its bulk of 10% formic acid and left from 3 to 5 minutes. It dies well extended. Transfer without washing to 1% Gold chloride (of commerce) for 25 minutes; then without washing into 1% formic acid for 24 hours, or until reduction is complete. This is indicated by a rich purple color over the whole specimen. Wash slightly in tap water; run up through the alcohols to chloroform; to chloroform saturated with hard paraffine. My sections are usually cut 16$\mu$ thick. When the impregnation appears to be very light—almost a failure, stain the sections on the slide with erythrosin or some other deep red anilin stain for contrast. These sections will often show the most exquisite details.

Transparent larvæ 5 to 10 mm. long require a milder treatment, such as the following: 5% formic acid 2 or 3 minutes, 1% or ½% gold chloride 10 minutes, weak formic 1 to 4 hours. If the specimens are watched from time to time under the dissecting lens, it will be seen that the central nervous system stains first and then the peripheral. The reduction of the gold chloride may be stopped, of course, at any point by transferring to alcohol.

[1]Edited by C. O. Whitman, University of Chicago.

All the operations described above were conducted in diffuse daylight and the gold chloride solution was exposed to sunlight for some time before using. This may not be an essential factor to the process, but Dr. L. Lindsay Johnson, in the third edition of Lee's *Vade Mecum*, suggests that failure to ripen the solution by sunning may be the cause of many of the failures in gold staining.

C. L. BRISTOL.

University of Chicago, April 14, 1894.

**Gold Chloride–Formic Acid Staining of Sections after Fixation in Sublimate Alcohol.**—S. Apathy in the the Zeitschrift für Wissenschaftliche Mikroskopie. Bd. X, 1893, p. 348.

The following method is extracted from an article on the muscle fibres of Ascaris.

Take equal parts of a saturated solution of corrosive sublimate in a $\frac{1}{2}$ per cent solution of common salt and absolute alcohol; or dissolve 3 per cent of corrosive sublimate and $\frac{1}{2}$ per cent common salt in 50 per cent alcohol. Use the liquid boiling hot for Ascaris, cold for leeches, and leave the animals in it for 24 hours, or at least 12 hours. Wash out in 50 percent alcohol until the mahogany-brown color of an iodine-alcohol solution remains unchanged for a few days. Free the tissues from iodine in 90 per cent alcohol. Imbed in paraffin, using chloroform for the transferring medium, and fix the sections on the slide. Free them completely from paraffine and chloroform, and finally wash slightly with distilled water.

Put the slide in a 1 per cent gold chloride solution and keep in the dark for 24 hours. Drain the slide and lightly apply a smooth-faced blotting paper to take up the surplus liquid. A $\frac{1}{10}$ per cent solution of gold chloride will answer, and is, of course, cheaper. Without further washing put the slide in a large bulk of 1 per cent formic acid and leave it for 24 hours. The longer diffuse daylight acts on the sections, the better the results. Wash in distilled water and mount in balsam. The sections may be cut very thin or thick—from $1\mu$ to $15\mu$, but the author found the best results from sections 2 or $2\frac{1}{2}\mu$ in thickness.

"By this simple procedure, founded on a well known method, are produced the most beautiful pictures of the finer details of various tissues, but especially muscle and nerve fibres. The various elements of the tissue are stained in different tints from rose to cherry red or red-brown and are sharply defined."

**A Rapid Method of Hardening and Sectioning.**[2]—Every practical pathologist must be convinced of the great importance, in many cases, of at once supplementing and completing the naked eye examination of structures by a thorough microscopic examination. Microscopic examination in the fresh state, by teasing up parts of tissues, or by means of scrapings from the cut surface, is in most cases imperative if the finer details of the cellular elements are to be fully appreciated, but sections are no less necessary in many cases if the relations of the various constituents, and the structure with the tissue as a whole, are to be determined. In order to do this the method of freezing the fresh tissue, and cutting sections with the microtome is frequently adopted, but it must be the general experience that such sections are often very unsatisfactory. They are so loose and lacking in cohesion, and the process of freezing alters the tissue so much, that they are difficult to manipulate and often difficult to interpret. I have occasionally met with errors in diagnosis made by incompetent observers from the use of such sections. In order to obtain satisfactory results, the processes of hardening, embedding, section-cutting, staining, and mounting are all necessary, and these commonly extend over several days. If the process can be so shortened that the whole investigation can be completed at one sitting, then a considerable practical advantage will be obtained. How often does it happen in the course of a pathological investigation of parts either obtained post-mortem or from operation that we wish to be satisfied on the spot as to the real significance of some particular appearance. If the structure is put aside to harden, there is considerable likelihood of some of the points being forgotten, and, at any rate, it is not taken up with the freshness of the first examination. I believe also that for purposes of surgical diagnosis an examination made within an hour's time would often be found of great value.

The method I have now to describe has no claim except as a practical working procedure. I have mentioned it to several friends, and have met with a general expression of its usefulness. I have used it constantly for more than a year, and am perfectly satisfied that it fulfils its purpose. The principles of the method are: (1) rapid hardening in alcohol; (2) cutting with the microtome without removing the alcohol and without freezing the tissue; (3) rapid staining.

1. The hardening is effected by absolute alcohol, kept at a temperature about that of the animal body. In examining the fresh tissue

[2]Journ. Pathology and Bacteriology, II, No. 4, May, 1894.

with the naked eye the pathologist makes up his mind as to what exact parts he desires to submit to microscopic examination. With a sharp knife he takes a thin slice of such a part, not more than two to four millimetres in thickness and of comparatively small superficial area. The piece of tissue is placed in a test-tube containing some cotton-wool at the bottom, and half-filled with absolute alcohol. The slice is so placed in the tube that it shall lie flat and not be distorted or curved. The vessel is now to be placed at a slightly elevated temperature, for which purpose a water bath is most suitable. I use a hand basin, the hot tap of which is left running so as to keep the water at a temperature which may be judged of by the hand. The slight current in the water is a distinct advantage. If the piece be at all bulky it may be well to renew the alcohol after a short interval. In the course of half an hour or three quarters the slice of tissue will generally be found sufficiently hardened to be proceeded with further.

2. In the next stage advantage is taken of the fact that anise-oil freezes at a comparatively high temperature (45° to 70° Fahr.), and that the presence of alcohol does not interfere with the process of freezing. My attention was called to this agent by a paper by Kühne. This author recommends anise-oil as an embedding material, but I have not found the method which he recommends very successful. I use the anise-oil, not to penetrate the tissue, like celloidin or paraffin, but rather to hold it and fix it on the plate of the microtome. Having taken the slice of tissue from the alcohol, I dry it with blotting-paper or an absorbent cloth. I then pour a few drops of anise-oil on the plate of the freezing microtome, and place the piece of tissue in the midst of the oil. It is better to have the oil making one convex drop with the specimens in the middle of it, as in cutting the sections the less oil you take with you the better. A few systoles of the ether-spray bellows suffice to freeze the oil into a white solid mass. The knife is now used with a considerable sweep, and the section may be cut dry if its superficial area be small. If this cannot be done without risk of tearing, then the upper surface of the blade may be moistened with alcohol. The microtome which I use for the purpose is a Schantze, and any microtome with a sliding knife will serve. It is possible, by this method, to obtain sections sufficiently thin for most purposes, although not equal, of course, to those which may be got after embedding in celloidin or in paraffine.

In regard to the size of the piece of tissue to be cut, it is certainly better to have it of small dimensions, but the method is perfectly applicable to such a piece as would involve, say, the whole thickness of the kidney including cortex and pyramids.

After the sections are made they are placed in alcohol, which dissolves the anise-oil.

The sections so obtained may be stained with any of the ordinary agents. I used Biondi's fluid a good deal; it is rapid and differentiates well. Perhaps the most generally useful stain is Mayer's carmalum. This has all the advantages (and they are many) of alum-carmine, and has some additional ones of its own. Thus it is much brighter in tint, and so forms a better contrast. This is of special service when Gram's or Weigert's method is used for the detection of microbes, as the blue tint of alum-carmine is often objectionable when the microbes are stained blue. I commonly use picric acid as a contrast stain with the carmalum. The solution used consists of alcohol seventy parts, saturated watery solution of picric acid 30 parts, and hydrochloric acid ½ part. I find the results obtained to be much better than those yielded by picrocarmine in my hands. The whole process of staining by carmalum and picric acid need not take many minutes. If necessary a gentle heat may be used to hasten the action. An excellent method of staining, in many respects, is that described by Nicolle. It is introduced as a method of staining microbes which do not stain by Gram's method. The staining agent is Kühne's or Sœffler's blue. I have used, chiefly, Kühne's blue, which acts very rapidly, a few seconds being usually enough. It is so very vigorous, that dilution is sometimes necessary. The section is then washed in water and treated with a 10 per cent. solution of tannic acid. This has the effect of fixing the blue color in nuclei and microbes, so that subsequent treatment with alcohol and oil of cloves will not remove the color. The section is taken from the tannin solution, washed in water, dehydrated with alcohol, cleared with oil of cloves, washed in xylol, and mounted in Canada balsam in the usual way. If a contrast stain he desired, then eosin or acid fuchsine may be added to the tannin solution.

To summarise the method it may be put as follows:

1. Select an illustrative part of the fresh tissue, and remove a slice with a sharp knife.
2. Place in absolute alcohol and heat the vessel in a water bath to about 40° C. for half an hour to an hour.
3. Dry the tissue and place on the freezing plate of the microtome in a large drop of anise-oil.
4. Freeze and cut sections. The upper surface of the knife may be moistened with alcohol while cutting.
5. Place in alcohol to remove anise-oil.
6. Float out in water and place on slide for staining.
7. Stain by any approved rapid method, and mount.—JOSEPH COATS, M. D.